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COMPARATIVE STUDIES OF THE NUTRITIONAL QUALITIES OF THREE FERMENTED AFRICAN LEGUME SEEDS USING *Bacillus subtilis* and *Bacillus pumilus* AS STARTERS

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ABSTRACT
Analysis into three African legume seeds; *Prosopis africana*, *Parkia biglobosa* and *Glycine max* was carried out. Unprocessed seeds of *Prosopis africana* were purchased from Otukpo market in Benue state of Nigeria and unprocessed seeds of *Parkia biglobosa* and *Glycine max* were purchased from Sabon-Gari market, Zaria, Kaduna state. These seeds were processed and fermented using 5% mixed *Bacillus subtilis* and *Bacillus pumilis* as inoculum into 300g of unfermented seeds. Seeds were allowed to ferment naturally along side with seeds inoculated with inoculum in the Department of Microbiology Ahmadu Bello University Zaria, for 48h. For *P. biglobosa* and *G.max, P.africana* seeds were left to ferment for 118h. Fermented seeds from the analysis showed that seeds with inoculum fermented faster giving higher protein and lipids values than seeds allowed to ferment naturally. Fermented seeds of *P. africana* were richer in protein with a crude protein value of 40.07% followed by *G.max* with a crude protein value of 38.29% and *P. biglobosa* with a crude protein value of 36.13%. Fermented seeds of *G.max* were richer in lipids with a crude lipid value of 18.42%, followed by *P.biglobosa* with a crude lipid value of 13.01% and *P.africana* with 12.01%. These seeds were fermented into "*daddawa*" and "*okpehe*" condiments as flavour enhancers. The analysis of variance showed that the moisture content, Ash, Protein and Carbohydrate content of the seeds vary significantly ($F < 0.0001$). On the other hand, the lipid and fibre contents did not vary significantly, ($F > 0.0001$).

KEYWORDS: *P.africana, P.biglobosa, G.max,* inoculum, protein, lipids, *"daddawa", "okpehe"*, condiments.

INTRODUCTION

Seeds of some legumes such as *Glycine max*, (soyabean seeds) *Prosopis africana*,(African mesquite) and *Parkia biglobosa* (locust bean seeds) may account for up to 80% of dietary protein. This may be the only source of protein for some group of people. Their cooked forms may be eaten as meals and are commonly used in fermented forms as condiments to enhance flavour of foods (Oniofiok, 1996). Fermented condiments remain key constituents of diet throughout many parts of Asia and Africa. Fermentation processes evolved for the development of taste and aroma, often result in enhanced nutrition and detoxification of antinutrient factors (Ogunshe *et al.,* 2006). Traditionally, fermented condiments (*"daddawa"* and *"okpehe"*) are based on vegetable proteins and are consumed by different ethnic groups in Nigeria. They serve not only as nutritious non-meat protein substitutes, but also as flavour enhancers in soups and other dishes (Achi, 2005). Traditional diet in West Africa consists of large quantities of staple foods (cassava, yam and maize). The staple foods provide the calories, but are poor in other nutrients. Soups are the main source of protein and minerals and one of the ways to improve the diet is to improve the nutrients in the soups (Achi, 2005).

Recent microbiological information on Nigerian plant protein fermentations includes, those reported by Omafuvbe *et al.,* 2002; Ouoba *et al.,* 2003; Dakwa *et al.,* 2005. In all their reports the involvement of genus *Bacillus* has been established in fermentation of locust bean seeds and other legumes. Fermentation in developing countries does not use inoculum, these processes could be improved by using starter cultures (Holpzapfel, 2002). A pure starter culture is essential for controlled fermentation. Controlled fermentation of soya beans was achieved by using pure single culture of *B. subtilis, B.licheniformis* or in combination (Sarkar *et al.,* 1993). The use of a mixture of microorganisms with complimentary physiological properties seems to be the best approach for obtaining a product with the nutritional and sensory properties desired

(Ouoba *et al.*, 2003). Therefore the objective of this study is to carry out fermentation of leguminous seeds using standard and test strains of *B.pumilus* and *B.subtilis* to compare the nutritional properties/proximate analysis.

MATERIALS AND METHODS:

Unprocessed samples of *P.africana* were purchased from Otukpo market, Benue state of Nigeria and samples of *G.max* and *P. biglobosa* were purchased from Sabon-Gari market, Zaria, Kaduna state of Nigeria. These seeds were transported to the laboratory, Department of Microbiology, Ahmadu Bello University, Zaria in polyethylene bags for processing.

Isolation and characterization of *Bacillus* species

Test strains of *B. pumilus* and *B. subtilis* isolated from fermented legume seeds were obtained from Department of Microbiology, Ahmadu Bello University, Zaria. Standard strains of *B. pumilus* and *B. subtilis* were obtained from Federal Institute of Industrial Research, Oshodi (FIIRO). These organisms were identified biochemically using various tests. These include the following biochemical tests as described by Gordon *et al.*, 1973. Indole test, coagulase test, motility test, catalase test, hydrogen sulphide production, starch hydrolysis, sugar fermentations and utilization, growth at different sodium chloride (NaCL) concentrations (5%,10%,15% 20% and 25%).

Preparation of *Bacillus* inoculum

The inoculum used for the fermentation contains 2.1×10^7 cells/ml, the cell population was calibrated using McFarland standard (No 7), and the amount used formed 5.0% for fermentation comprising of 15ml of 24h old cultures of organism consortium A (Standard strains of *B.subtilis* and *B.pumilus* combined) and consortium B (Test strains of *B.subtilis* and *B.pumilus* combined)

Controlled Fermentation of locust bean, soyabean and African mesquite seeds

The fermentation process was set up using both organism consortiums respectively. These organisms were inoculated into 300g of processed unfermented seeds. The seeds were wrapped with sterile aluminum foil paper and placed in an earth pot with cover. The fermentation was allowed to progress at room temperature (28 ± 2^0C) for 48h for *P.biglobosa* and *G.max*, 118h for *P.africana* in the Department of Microbiology, Ahmadu Bello University Zaria. After fermentation, proximate analyses for crude protein, carbohydrates, crude lipids, fiber, ash and moisture content were carried out adopting the AOAC (1980) methods.

Analysis of results

The data were analyzed for significance using Analysis of Variance, by the Statistical Analysis System version 12.

RESULTS

The moisture and ash content were higher in unfermented seeds of *P.biglobosa* with values of 67.50% than in other seeds. Crude lipids were higher in unfermented seed of *G.max* with a value of 10.00% than other seeds. Crude protein in unfermented seeds of *P.africana* was higher with a value of 17.13% than other seeds. Crude fiber value in unfermented *P.africana* seeds was much lower with a value of 0.01% while *P.biglobosa* and *G.max* had fiber values of 0.38%. Soluble carbohydrate was higher in unfermented seeds of *P.biglobosa* than the rest of the seeds with a value of 10.63%. The analysis of variance showed that the moisture content, Ash, Protein and Carbohydrate content of the seeds vary significantly ($F < 0.0001$). On the other hand, the lipid and fibre contents did not vary significantly, ($F > 0.0001$).

The results of the fermented seeds with the organism (consortium A and B) are also presented in Table 1. The moisture content of seeds of *P.africana* and *P.biglobosa* fermented with consortium A and consortium B was 65.00% respectively, while moisture content was lower in fermented seeds of *G.max* subjected to the same condition, with a value of 62.00% respectively. Ash content was higher in consortium B fermented seeds of *P.africana* with values of 2.30%, and lower in consortium B fermented seeds of *G.max* with values of 0.01%. During fermentation, crude lipid and crude protein increased significantly as shown in Table 1.

Table 1: Proximate Analysis of Unfermented, Naturally Fermented and starter assisted Fermented Seeds of *P.africana, P. biglobosa* and *G. max* (%)

Nutrients	State of the seeds											
	Unfermented seeds of			Naturally fermented seeds			Fermented seeds assisted with starters					
	P. africana,	*P. biglobosa* &	*G. max*	NFS4	NFS5	NFS6	FSSS1	FSTS1	FSSS2	FSTS2	FSSS3	FSTS3
Moisture content	* 65.30 ± 0.26	67.50 ± 0.00	62.30 ± 0.30	65.0 ± 0.50	62.20± 0.27	62.20± 0.27	65.10 ± 0.10	65.30± 0.20	65.30± 0.20	65.43± 0.21	62.20± 0.26	62.13± 0.15
Ash	1.44 ± 0.00	1.93 ± 0.03	0.46 ± 0.01	2.30 ± 0.00	2.50 ± 0.01	0.22 ± 0.00	2.15± 0.12	2.30 ± 0.00	1.51± 0.01	1.52 ± 0.02	0.04 ± 0.50	0.01 ± 0.50
Crude lipid	2.55 ± 0.02	2.52 ± 0.02	1.91 ± 0.01	11.96 ± 0.01	12.74 ± 0.01	17.43 ± 0.01	12.03 ± 0.05	12.01 ± 0.00	13.03 ± 0.02	13.00 ± 0.00	18.43 ± 0.01	18.43 ± 0.01
Crude protein	17.13 ± 0.01	11.53 ± 0.01	15.81 ± 0.00	38.70 ± 0.20	36.00 ± 0.00	38.03 ± 0.05	40.05 ± 0.01	40.06 ± 0.00	36.14 ± 0.01	36.13 ± 0.01	38.27 ± 0.02	38.29 ± 0.01
Crude fiber	0.01 ± 0.00	0.36 ± 0.01	0.38 ± 0.01	0.51 ± 0.00	1.50 ± 0.00	0.2 1± 0.01	0.38 ± 0.00	0.38 ± 0.01	1.92 ± 0.00	1.93 ± 0.00	0.03 ± 0.01	0.02 ± 0.01
Soluble carbohydrate	4.04 ± 0.02	10.63 ± 0.01	4.31± 0.00	0.15 ± 0.00	12.09 ± 0.07	7.83 ± 0.00	0.17 ± 0.01	0.17 ± 0.01	7.99 ± 0.00	7.95 ± 0.02	1.82 ± 0.02	1.81 ± 0.00

*All values are means of triplet readings. The analysis of variance showed that the moisture content, Ash, Protein and Carbohydrate content of the seeds vary significantly ($F < 0.0001$). On the other hand, the lipid and fiber contents did not vary significantly, ($F > 0.0001$).

Keys
NFS4 - Naturally fermented seeds of *P. africana*
NFS5 - Naturally fermented seeds of *P.biglobosa*
NFS6 - Naturally fermented seeds of *G.max* .
FSSS1 - Fermented seeds of *P.africana* with consortium A.
FSTS1 - Fermented seeds of *P.africana* with consortium B.
FSSS2 - Fermented seeds of *P.biglobosa* with consortium A
FSTS2 - Fermented seeds of *P.biglobosa* with consortium B.
FSSS3 - Fermented seeds of *G.max* with consortium A.
FSTS3 - Fermented seeds of *G.max* with consortium B.

Values of crude lipids were higher in both consortiums fermented seeds of *G.max* with a value of 18.43% and lower in consortium A and B fermented seeds of *P.africana* with values of 12.03% and 12.01% respectively. Protein value was higher in consortium B and A, fermented seeds of *P. africana* with values of 40.06% and 40.05% followed by consortium B fermented seeds of *G.max* with values of 38.29% and consortium A fermented seeds of G.max with values of 38.27%. Percentage fiber was higher in consortium B fermented seeds of *P.biglobosa* with values of 1.93% and lower in consortium B fermented seeds of *G.max* with values of 0.02%. Soluble carbohydrate was higher in consortium A fermented seeds of *P.biglobosa* with values of 7.99% and consortium B with values of 7.95% and lower in consortium A and B fermented seeds of *G.max* with a value of 1.81%. Nutritional information of *P.africana* seeds fermented naturally had a higher moisture content of 65.00%, while those of *P.biglobosa* and *G.max* had a moisture content of 62.20%. Values of ash content were higher in naturally fermented seeds of *P.biglobosa* which had 2.50% and lower in *G.max* with a percentage value of 0.22%. Crude lipids value was higher in naturally fermented seeds of *G.max* with a value of 17.43% and lower in naturally fermented seeds of *P.africana* with a value of 11.96%. Crude proteins were higher in naturally fermented seeds of *P.africana* with a value of 38.70% and *G.max* with a value of 38.03% and lower in naturally fermented seeds of *P.biglobosa* with a value of 36.00%. Crude fiber was higher in naturally fermented seeds *P.biglobosa* seeds with a value of 1.50% and lower in *G.max* with a value of 0.21%. Values of soluble carbohydrate were higher in naturally fermented seeds of *P.biglobosa* with a value of 12.09% and lower in naturally fermented seeds of *P.africana* with a value of 0.15%

DISCUSSION

Fermentation remains an effective, inexpensive method for extending the shelf life of foods and increasing their nutritional content (Tamang, 2009). Lipids content and protein increased significantly as shown in Table 1. This is due to the fact that fermentation results in increased nutritional values which are in agreement with the results of Odunfa (1984). In Table 1, the three legume seeds fermented with both consortiums gave higher values of lipids and proteins than those fermented naturally. This is due to the fact that when organisms responsible for legume seeds fermentation are used as inoculum or starters in the right percentage, it will guarantee consistency and product quality (Holpzapfel, 2002). Seeds fermented without starters had higher moisture content, higher fiber and carbohydrates values. Protein values were low compared to seeds fermented with starters. Inconsistencies in naturally fermented seeds aroused because there was no appropriate starters to initiate fermentation. The use of consortium A and B for fermentation resulted in products of high nutritional properties desired which is in agreement with Holpzapfel 2002.

CONCLUSION

It was concluded from this research that fermented legume seeds have higher nutritional values than unfermented legume seeds. Seeds of *P.africana*, *P.biglobosa* and *G.max* fermented with consortium A and B as starters gave higher nutritional values than seeds allowed fermenting naturally. Therefore, legume seeds fermented with both consortiums can be use as condiments and flavour enhancers in soups and other dishes.

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